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Isolation and Characterization of Soluble Proteins from Moringa (Moringa Oleifera Lam) Seeds for Possible Use in Food Industries

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ABSTRACT

Background: Proteins are crucial for the functional properties and sensory characteristics of foods. This study aims to isolate and characterize soluble proteins from *Moringa oleifera* seeds. **Methods:** The seeds were defatted using chloroform, methanol, and water (1:2:0.8). Protein isolates were prepared by aqueous extraction, 0.1M NaOH precipitation, and 60% $(NH_4)_2SO_4$ precipitation. Moreover, proximate composition of the dry seeds, and the protein yield, physicochemical properties, functional properties, and amino acid composition of the isolates were determined using standard methods. **Results:** 100 g of dry seed contained 29.09±0.40 g proteins. The aqueous extract had the greatest protein content (73.0±0.5 g/100g), followed by NaOH (54.0±0.2 g/100g) and $(NH_4)_2SO_4$ (45.0±0.3 g/100g). As a result, the aqueous extract showed a significantly (P<0.05) maximum *in vitro* digestibility (82.63±0.27g) after 6 hours. Strong UV absorption at 280 nm was recorded for all the protein isolates. All essential amino acids were present, and phenylalanine was the chief amino acid (8.93±0.67 mg/100g in $(NH_4)_2SO_4$ fraction) which was significant at P<0.05. NaOH and $(NH_4)_2SO_4$ fractions were most soluble at pH 10 which were significant at P<0.05. The NaOH fraction had the peak foaming capacity (28.0±0.4%) at pH 10, and the most stable foam was observed in the NaOH fraction after 10 minutes. Significantly (P<0.05) elevated in the $(NH_4)_2SO_4$ fraction (32.0±0.6%), followed by aqueous extract (28.0±0.5%) at pH 10 was the emulsifying capacity. The $(NH_4)_2SO_4$ fraction had significantly (P<0.05) more water-holding capacity (2.80±0.04 g/g); the prime oil-holding capacity was also observed in the aqueous extract (3.20±0.02 g/g). **Conclusion:** *Moringa oleifera* seeds possess essential amino acids and functional properties beneficial for food systems, potentially enhancing value or developing new products.

Introduction

Moringa oleifera (M. oleifera), also called horseradish or drumstick, is a tropical plant native to India and is cultivated all year round in Asia, sub-Saharan Africa, and Latin America (Vijayaraghvan *et al.*, 2011). The seeds, leaves, bark, and roots of this plant possess numerous

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applications in nutrition, medicine, and commercial purposes (Anwar *et al.*, 2007, Lakshmipriya *et al.*, 2016). Earlier researchers found that *M. oleifera* seed is not toxic (Bakare, 2016, Yuliastri *et al.*, 2016). The seed of this plant is very common and easy to store, especially in developing countries (Sánchez-Martín *et al.*, 2012). Ndabigengesere *et al.* argued that the active agents of *M. oleifera* are found in the seed kernel rather than seed bark (Ndabigengesere *et al.*, 1995). The seed kernel was reported to possess significant quantities of a series of low molecular weight, water-soluble proteins that carry an overall positive charge in solution (Jayalakshmi *et al.*, 2017).

There has been a growing and strong interest in plant proteins; so, the authors turn to alternatives such as plant-based proteins which have reduced carbon footprint, higher sustainability, lower price, and non-allergenicity (Jain *et al.*, 2019). Plant-based proteins are considered vegan food, provide an ample number of amino acids, are directly absorbed by the body, and help in treating various disease ailments. Successful functional plant-based protein product is the soy protein which includes soy protein isolate, soy protein concentrate, and textured soy protein (Singh *et al.*, 2008). Nevertheless, there are people who are allergic to soy protein and need an alternative to soy as well as animal proteins ;also, there is a strong interest in evaluating other plant proteins besides soy (Małecki *et al.*, 2021). *M. oleifera* seeds represent another source of plant-based proteins with potentially important functional properties that could enhance the structure, texture, and organoleptic properties of food products. This study aims at isolating and characterizing some soluble proteins from *M. oleifera* seeds.

Materials and Methods

Sample collection and preparation of M. oleifera seed

Dry *M. oleifera* seeds were purchased from Gamboru local market, Maiduguri, Nigeria. The seeds were identified by a plant taxonomist at the Department of Biological Science, University of Maiduguri, and a Voucher specimen was deposited at the herberium. The seeds were ground into flour using mortar and pestle and were stored in a clean air-tight container for further analysis.

Proximate composition of M. oleifera seeds

The proximate composition of the seeds of *M. oleifera* was carried out using the standard procedures of the Association of Official Analytical Chemists (Association of Official Analytical Chemists (AOAC), 2005) on a dry basis.

Extraction and isolation of M. oleifera seed proteins

The ground powdered *M. oleifera* seeds (110.5 g) were defatted using a mixture of chloroform, methanol, and water in a ratio of 1:2:0.8. This was homogenized in the solvent to extract all lipids, more water was added to the resulting extract, and the mixture was separated into two phases, methanol/water (top phase) and chloroform (bottom phase). The lipids remaining in the chloroform layer were separated from the extract mixture, while the seed proteins in the methanol/water layer were collected and allowed to air dry. The dried seed extract was divided into three portions. The first portion was resolubilized and was considered as the aqueous extract containing seed proteins from *M. oleifera* seeds. The second portion was prepared by precipitating the proteins with 0.1 M NaOH (1:10, w/v), continuously stirred for 60 min, and subsequently centrifuged at 10000 rpm for 30 minutes. The seed meal was collected, air-dried and then stored in a clean container. The third portion was similarly prepared by the addition of 60% $(NH_4)_2SO_4$ with continuous stirring for 3 hours and subsequently centrifuged at 10000 rpm for 30 minutes and air-dried and stored in a clean container to obtain the isolate. Protein yield of the isolates were determined as described by Jain *et al.* using the following formula (Jain *et al.*, 2019):

$$Protein\ isolate\ yield\ (\%) = \frac{weight\ of\ protein\ in\ the\ fraction}{weight\ of\ defatted\ moringa\ seeds} X\ 100$$

Determination of in vitro protein digestibility

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In vitro digestibility was determined according to the method described by (Association of Official Analytical Chemists (AOAC), 2015). One millilitre of 11% Trypsin was introduced into 2 test tubes. Subsequently, 4 ml of phosphate buffer with pH 7.5 was added to each test-tube and 1 ml of 0.1 N HCl was also added and allowed to stand to equilibrate, after which 1 ml of 1% sample was added to all the test tubes (labelled as digestibility at 1 and 6 hrs). The reaction in each test tube was stopped with 5 ml of neutralized formalin at 60 min and 6 hrs. The content of the test tubes were then filtered using filter paper. The filter papers were dried in an oven at 108°C for 3 hr. The nitrogen of the undigested sample was determined by the Kjeldahl method:

$$Percentage\ in\ vitro\ digestibility = (CP1 - CP2)/CP1 \text{ x } 100$$

Where CP1 = Total protein of sample;
CP2 = Total protein after digestion with trypsin.

Ultraviolet absorption spectra of protein isolate

After being dissolved in 100 ml of deionized water, the protein was scanned using a UV-visible spectrophotometer in the range of 240 nm to 310 nm (Senthilraja, 2011).

Amino acids analysis

The defatted samples were utilized to estimate amino acids as described by (Association of Official Analytical Chemists (AOAC), 2000). The defatted samples were utilized to estimate amino acids. The sample (30mg) was then hydrolysed with 6N HCI at 110 °C for 24h. Amino acid analysis was performed on reverse phase-high pressure liquid chromatography (HPLC) (Buck scientific BLC 10/11 USA) equipped with UV 338nm detector. A C18, 2.5 x 200mm, 5um column and a mobile phase of 1:2:2 (100mM sodium phosphate, pH 7.2: Acetonitrile: methanol) was used at a flow rate of 0.45 mL/minute and an operating temperature of 40 °C. Mixed standards were analyzed in a similar manner for identification. After that, peak identification was conducted by comparing the retention times of authentic standards and those obtained from the samples, these data were later integrated using peak simple chromatography data system processor; (Buck SCi. chromatopac data processor).

Determination of functional characteristics

Protein solubility: The solubility of the protein was conducted according to the method described by Kumar *et al.* (Kumar et al., 2014). The sample (100 mg) was dispersed in 5 ml of distilled water. The pH of the mixture was adjusted to 4, 6, 8, 10, and 12 using 0.1 M HCl or 1 M NaOH. Then, the solution was stirred for one hour at room temperature and centrifuged at 4,000 x g for 20 minutes. The percentage solubility of the proteins was calculated as follows:

$$Solubility\ (\%) = \frac{\text{Amount of nitrogen in the supernatant}}{\text{Amount of protein in the extract}} \text{ x } 100$$

Foaming capacity (FC) and stability (FS): Foaming capacity was measured according to the method described by Lamsal *et al*. (Lamsal et al., 2007). The sample (500 mg) was added into a beaker containing 50 ml of distilled water, and the pH was adjusted to 4, 6, 8, 10, and 12 with either 0.1 M NaOH or 0.1 M HCl. The solution was whipped using the maximum speed of a homogenizer for 5 min and transferred into a 100 ml graduated cylinder while the volume of foam (V2) was immediately recorded at 0 min. The foam stability was determined by measuring the decrease in the volume of foam as a function of time up to a period of 10, 20, 30, and 40 min.

$$FC\ (\%) = \frac{V2}{V1} \text{ x } 100$$

Where V2 is the volume after whipping and V1 is the volume before whipping

$$FS\ (\%) = \frac{\text{Volume after standing} - \text{Volume before standing}}{\text{Volume before whipping}} \text{ x } 100$$

Emulsifying capacity (EC) and stability (ES): Emulsifying capacity was determined using the method described by Lamsal *et al*. (Lamsal et al., 2007). The sample (300 mg) was homogenized for 1 min in 20 ml distilled water, and the pH was adjusted to 3, 4, 6, 8, 10, and 12. The protein solution was mixed with 15 ml of soybean oil followed by homogenising for 1 min. It was then

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centrifuged at 5,000 rpm for 3 min. EC was calculated using the expression below.

$$EC\ (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of total content}} \text{ x } 100$$

Emulsifying stability was determined by heating the emulsion at 70 °C for 30 min in a water bath, after which, it was centrifuged at 5,000 rpm for 3 min.

$$ES\ (\%) = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \text{ x } 100$$

Water holding capacity (WHC) and oil holding capacity (OHC): WHC and OHC were determined as described by Mariod *et al*. (Mariod et al., 2010). The sample (0.5 g) was weighed into a 50 ml pre-weighed centrifuge tube and mixed with 10 g of distilled water for WHC determination, while 1.0 gram was mixed with 10 g of soybean oil for OHC determination. Then, the obtained suspensions were vortexed for 2 min and were left to stand for 30 min. The protein-water mixture was centrifuged at 3,000 g for 15 min. The supernatant was removed and the tube was then re-weighed.

$$WHC\ or\ OHC\ (g/g) = \frac{W2 - W1}{W1}$$

Where W1 is the weight of the tube plus the dry sample and W2 is the weight of the tube plus sediment.

Data analysis

All experiments were performed in triplicate. The results were presented as mean±standard deviation. Moreover, a two-way analysis of variance (ANOVA) with a post hoc test was used to detect statistical differences, particularly in functional properties, and analysis was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). The significance level was based on the confidence level of 95% (P<0.05).

Results

Table 1 presents the proximate composition of the dry seeds of *M. oleifera*. The seed contains high fats (46.41±0.80 g/100 g), followed by proteins (29.09±0.40 g/100 g). The least amount of component regarding the seed was (3.27±0.01 g/100 g) which represents the soluble carbohydrates.

Table 1. Proximate composition of *M. oleifera* per 100 g of dry seeds.

Component	Amount
Moisture	6.70±0.05[a]
Ash	4.45±0.03
Protein	29.09±0.40
Fat	46.41±0.80
Fibre	10.08±0.04
Carbohydrate	3.27±0.01

[a]: *Mean ± SD.*

Table 2 shows the protein content of the isolates. The aqueous extract contained 73.0±0.5 g/100 g protein, while the NaOH precipitate fraction and the $(NH4)_2SO_4$ fraction had 54.0±0.2 and 45.0±0.3 g/100 g protein.

Table 2. Protein yield of *M. oleifera* seed isolates (g/100 g).

Isolate	Amount
Aqueous extract	73.0±0.5[a]
NaOH precipitate fraction	54.0±0.2
$(NH_4)_2SO_4$ precipitate fraction	45.0±0.3

[a]: *Mean ± SD.*

The results of the *in vitro* protein digestibility of the aqueous extract, the alkaline precipitated extract, and the ammonium sulphate precipitated extracts are presented in **Table 3**. The results indicated that a high digestibility value was recorded for the aqueous extract after 6 hours of digestion (82.63±0.27). In contrast, the alkaline precipitated fraction (76.85±0.61) and the ammonium sulphate precipitated fractions (77.91±0.12) had higher values than the aqueous extract after 1 hour of digestion.

The UV absorption spectra of the *M. oleifera* seed protein isolates are illustrated in **Figure 1**. The three protein isolates had shown strong absorption at 280 nm which indicates that protein is present in all the protein isolates.

The amino acid composition of the *M. oleifera* seed protein isolates are shown in **Table 4**. The essential amino acids are all present in the seed, with histidine having the lowest content and phenylalanine the highest amount 8.93 mg/100g in the $(NH_4)_2SO_4$ fraction.

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Figure 2 describes the solubility of the protein isolates. The solubility of all the fractions was higher at alkaline pH values. The NaOH precipitated fraction and the ammonium sulphate fraction are more soluble than the aqueous extract.

Table 3. *In vitro* digesytibility of *M. oleifera* seed soluble protein isolates.

Samples	Amount in grams of protein digestibility at 1 hour	Amount in grams of protein digestibility at 6 hours
Aqueous extract	74.91±0.32[a]	82.63±0.27[a]
NaOH precipitate	76.85±0.61[a]	68.17±0.42[b]
$(NH_4)_2SO_4$ precipitate	77.91±0.12[a]	68.78±0.25[b]

Values with different superscript along a column are significantly different (P<0.05).

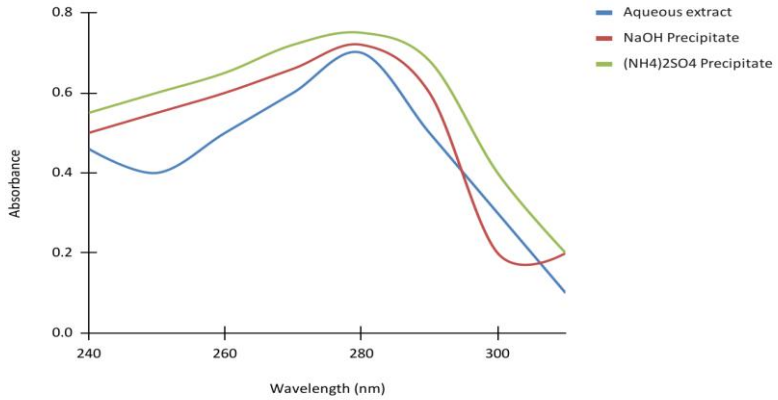

Figure 1. Ultraviolet absorption spectra of *M. oleifera* seed protein isolates.

Table 4. Amino acids composition of *M. oleifera* seed protein isolates (mg/100g).

Amino acid	Crude extract	NaOH precipitate	$(NH_4)_2SO_4$ precipitate
Tryptophan	1.54±0.10	2.62±0.23[a]	4.17±0.35[b]
Histidine	0.855±0.02	1.13±0.01	1.32±0.12
Leucine	1.745±0.01	2.25±0.22	3.65±0.62[a]
Isoleucine	2.01±0.12	2.84±0.51	4.04±0.51[a]
Phenylalanine	6.25±0.23	7.34±0.36	8.93±0.67[b]
Valine	2.75±0.03	3.84±0.51[a]	3.87±0.44
Lysine	3.87±0.41	4.01±0.23	4.26±0.63[b]
Methionine	2.72±0.22	3.78±0.41[a]	5.26±0.81[b]
Threonine	1.535±0.14	1.87±0.02	3.85±0.46[a]
Asparagine	3.61±0.02	4.23±0.64	6.32±0.35[a]
Arginine	2.80±0.30	3.13±0.26	3.45±0.23
Alanine	0.56±0.01	0.71±0.02	0.98±0.23[b]
Aspartate	3.23±0.25	4.62±0.51[a]	5.81±0.63[b]
Glutamate	1.77±0.11	2.71±0.32[a]	4.15±0.24[b]
Glycine	4.57±0.43	6.52±0.56[a]	7.34±0.45[b]
Tyrosine	4.43±0.35[a]	3.14±0.32	4.11±0.75[a]
Cysteine	3.13±0.12	3.41±0.41	6.05±0.86[a]
Proline	1.71±0.50	1.83±0.15	2.29±0.54
Serine	2.75±0.32	3.12±0.04	4.84±0.63[a]

Values are expressed as mean ± SD (n = 3); Values with different superscripts across a row are significantly different (P<0.05)..

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The results for the foaming capacity and foaming stability are depicted in Figure 3 and Figure 4 respectively. The fraction for the NaOH precipitate fraction had the highest foaming capacity (28%) at pH 10. Similarly, the highest foaming capacity for the ammonium sulphate precipitated fraction (20%) was observed at pH 10.

Figure 5 presents the emulsifying capacity of *M. oleifera* seed protein isolates. The highest emulsifying capacity was observed with the $(NH_4)_2SO_4$ fraction.

Figure 6 shows the emulsifying stability of *M. oleifera* seed protein isolates. The aqueous extract is the most stable at both pH 10 and pH 12. The $(NH_4)_2SO_4$ fraction is also significantly (P<0.05) stable at pH 12 and pH 10 with stability of 45% and 40% respectively.

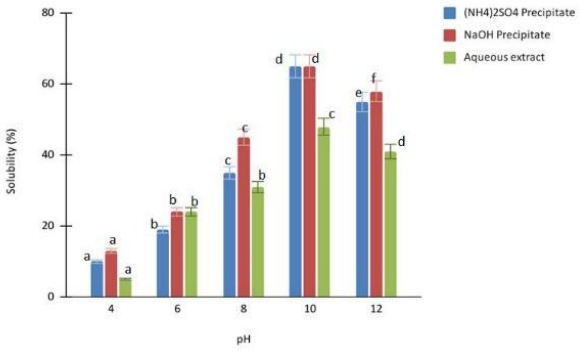

Figure 2. Solubility of *M. oleifera* seed protein isolates Same color bars with different letters above at different pH are significantly different (P<0.05).

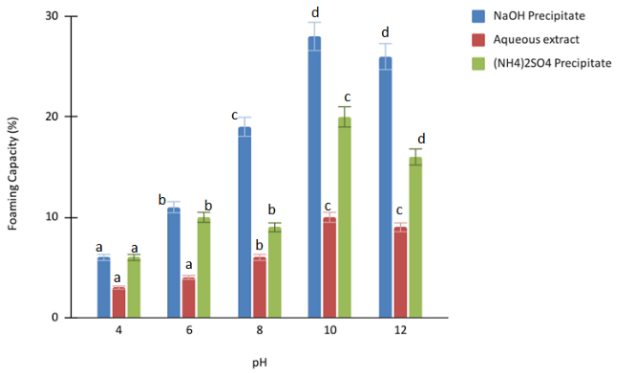

Figure 3. Foaming capacity of *M. oleifera* seed protein isolates Same color bars with different letters above at different pH are significantly different (P<0.05).

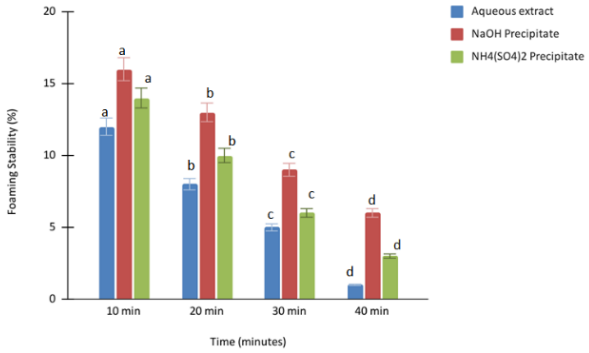

Figure 4. Foaming stability of *M. oleifera* seed protein isolates. Same color bars with different letters above at different times are significantly different (P<0.05).

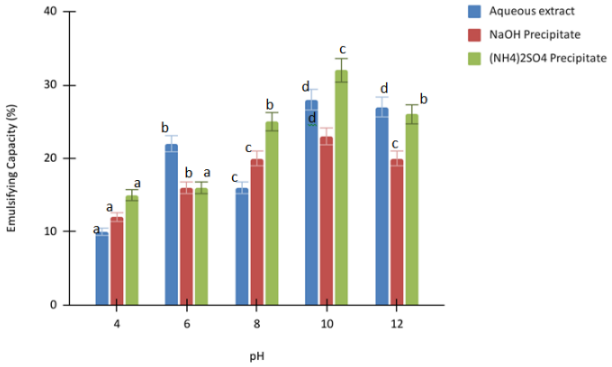

Figure 5. Emulsifying capacity of *M. oleifera* seed protein isolates. Same color bars with different letters above at different pH are significantly different (P<0.05).

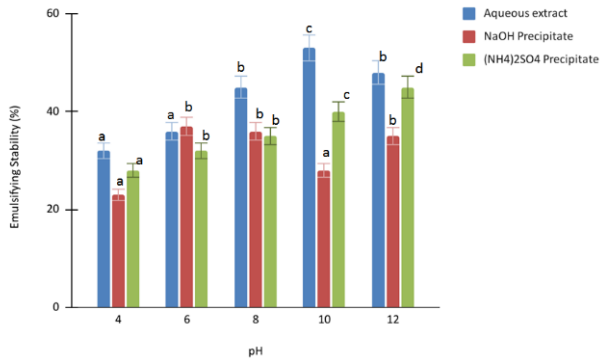

Figure 6. Emulsifying stability of *M. oleifera* seed protein isolates. Pame color bars with different letters above at different pH are significantly different (P<0.05).

The water-holding capacity and oil-holding capacity of *M. oleifera* seed protein isolates are shown in **Figure 7**. The results showed that

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$(NH_4)_2SO_4$ fraction had a higher water-holding capacity (2.8 g/g) than the aqueous extract and the NaOH fractions. The fraction with the most oil-holding capacity was the aqueous extract (3.2 g/g).

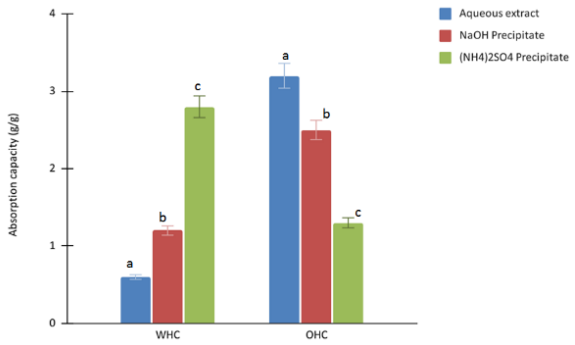

Figure 7. Water-holding capacity and oil-holding capacity of *M. oleifera* seed protein isolates
Bars with different letters above for WHC and OHC respectively are significantly different (P<0.05).

Discussion

There is an increased awareness of the consumption and use of *M. oleifera* seeds in food systems in the tropics (Ogunsina *et al.*, 2010). The results of the proximate composition was similar to the findings of Zaku *et al*. where they reported a protein content of 27.1% per 100 g sample (Zaku *et al.*, 2015). A profile of 30.07% and 33.26% of protein has been reported for mature seeds from *M. oleifera* cultivated in Pakistan and Brazil, respectively (Manzoor *et al.*, 2007, Oliveira *et al.*, 1999). These variations might have to do with differences in the plant's maturation stage, geographical distribution, post-harvest handling, ambient conditions, and processing methods, all of which can affect the nutritional value of *M. oleifera* plant parts (Iyapo and Omotosho, 2024).

The observation of the total amino acid content revealed that the essential amino acids tryptophan, leucine, isoleucine, phenylalanine, valine, lysine, methionine, and threonine are available in the range of 3.6 - 8.9 mg/100g of protein (**Table 4**). It can be vividly noticed that the fraction from the $(NH_4)_2SO_4$ precipitate contained higher concentrations of the amino acids, compared with aqueous extract and the NaOH precipitate fraction. It was reported that all the parts of this plant contain high percentages of essential amino acids except for methionine, commonly deficient in green leaves (Sánchez-Machado *et al.*, 2010).

While amino acid composition reveals the nutritional quality of protein food, especially the quantity of each amino acid present, protein digestibility shows the utilization potential of such protein (Boye *et al.*, 2012). The decrease in pH during protein digestibility is due to the release of hydrogen ions by the hydrolyzed peptide bonds. A more rapid decrease, which signifies an increased rate of digestion, can be taken as an index of protein digestibility (Malomo and Aluko, 2015). The protein digestibility for the protein isolates shown in **Table 2** indicated a high protein digestibility value which might be attributed to the ease of access of the protease to the peptide bonds aided by the lower amount of non-protein materials (Adebowale *et al.*, 2007). In this respect, the $(NH_4)_2SO_4$ fraction was more digestible, which suggests a conformation with better access to enzymes when compared to the NaOH fraction and the aqueous extract. The values obtained in this study were comparable to 84% and 85% reported for soy protein concentrates and isolates, respectively (Mohamed *et al.*, 2009); however, lower than the 91-95% obtained for mucuna bean protein isolate (Adebowale *et al.*, 2007). Similar values have also been reported such as 68% for flaxseed (Marambe *et al.*, 2013) and 71% for pinto bean (Tan *et al.*, 2014).

Solubility characteristics of proteins are among the most important functional properties since many functional performances of proteins depend upon their capacity to go into solution initially. Once the pH is close to the protein isoelectric point (around pH 4−5), there is a reduction of solubility, as found by Mune-Mune *et al*., while above/below the protein isoelectric point, the more protein solubility was observed. (Mune Mune *et al.*, 2016). This might be the result of changes in aggregation, solubility, and secondary structure (Amiri *et al.*, 2021). Seena and Sridhar mentioned that at highly acidic and alkaline pH, the protein acquires, respectively, a net positive and negative charge, which promotes the repulsion of molecules, and

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thus, increases the solubility of the protein (Seena and Sridhar, 2005). This result has been described in many food proteins according to the tendency of protein solubility as a function of pH, including microalgae proteins, mung bean proteins, and sunflower proteins, which have a minimum solubility around an isoelectric point between a pH of 4–5 (Chen *et al.*, 2019, Du *et al.*, 2017, Ulloa *et al.*, 2017). Tang *et al.* reported the solubility of native water soluble protein from *M. oleifera* to be 5.56% where ultrasonic treatment increased the solubility significantly to 30.53% (Tang *et al.*, 2021).

Foaming capacity and foaming stability are important functional properties of protein isolates that determine their utilization in different food systems where aeration and overrun are required, for example, whipped toppings, baked foods, and ice-cream mixes (Shevkani *et al.*, 2015). High protein solubility is a prerequisite for achieving better foaming capacity (Deng *et al.*, 2011). Protein molecules should form continuous intermolecular polymers enveloping the air bubble to render good foaming stability since intermolecular cohesiveness and elasticity are important to produce stable foams (Kamara *et al.*, 2009). The higher foam stability observed (**Figure 4**) after 10 minutes could be attributed to the unfolding of the protein structure which facilitates surface hydrophobic association as well as reduced air leakage leading to the prevention of rupture and coalescence (Shevkani *et al.*, 2015, Wang *et al.*, 2012).

The emulsifying capacity is a measure of the effectiveness of proteinaceous emulsifiers (Pearce and Kinsella, 1978). The emulsifying capacities of the protein are generally dependent on its ability to absorb on the interface whereas emulsion stability is related to the stability properties of this adsorbed layer. The nature of the amino acids in each of the fractions could differ depending on the pH of protein resolubilization which might be the key factor affecting the emulsifying properties of the protein isolates (**Figure 6**) as well as other functional properties. Protein solubility and hydrophobicity are two major important factors that determine their initial adsorption, and thus, their emulsifying properties (Shevkani *et al.*, 2015).

It was reported that *M. oleifera* seed protein managed to hold 4.78 ml water/g protein (Paramita *et al.*, 2024). However, the poor water absorption capacity of the aqueous extract may not restrict its application because the salt content in the functional foods formulation would be sufficient to provide the desired solubility. Arrese *et al.* studied the functional properties of several commercial soy protein isolates and reported that isolates with more denaturation showed a greater water-holding capacity (Arrese *et al.*, 1991). This may be attributed to the unfolding of the polypeptide chain resulting in a matrix that can trap the absorbed water.

The differences in oil absorption capacity might be due to the presence of more non-polar amino acids in the aqueous extract. The presence of several non-polar side chains may bind the hydrocarbon chains of fats, thereby resulting in higher absorption of oil (Sathe *et al.*, 1982). The high oil absorption capacity of *M. oleifera* seed proteins makes them good ingredients in the cold meat industry, particularly for sausages, where the protein usually bridges the fat and water to obtain good products.

The scope of the study was limited to the isolation and partial characterization of some soluble proteins from the seeds of *M. oleifera* for use in food industries. This study did not involve any drugs or patient. No laboratory animal was used in the study. This study exclusively examined the properties of protein isolates precipitated with NaOH and ammonium sulfate, without subsequent dialysis to eliminate Na+ and ammonium sulfate residues from the protein isolates. Developing protein supplements or meat alternatives using *M. oleifera* seed protein isolates, especially the $(NH_4)_2SO_4$ fraction are recommended for improved digestibility. Incorporating *M. oleifera* proteins into emulsified products such as dressings or spreads is advised. They should be incorporated into baked goods or whipped toppings due to foaming properties and combined with other proteins to balance amino acids, addressing methionine deficiency.

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Conclusion

The high solubility, foaming, and emulsifying properties of *M. oleifera* seed proteins at alkaline pH levels might play an important role in food systems, thereby improving the quality or texture of foods. Similarly, good *in vitro* digestibility, further supported by the significant amounts of both dispensable and indispensable amino acids, suggests the possibility of using it as an alternative nutritious plant-based protein.

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Authors' contributions

Abdulrahman AA, Milala MA, Shehu BB, and Gidado A designed the research; Abdulrahman AA and Damasak AA conducted research; Abdulrahman AA, Buba F, and Tijani Y and Musa H analyzed data and drafted the manuscript; Milala MA, Shehu BB, and Gidado A edited the paper. Abdulrahman AA had the primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of interest

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